View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Kyoto University Research Information Repository

KURENAI
Kyoto University Research Information Repository

KYOTO UNIVERSITY

Title	Determination of Serum Tocopherols by High Performance Liquid Chromatography
Author(s)	KATO, HITOSHI; TANIMURA, HIROSHI; HIKASA, YORINORI
Citation	日本外科宝函 (1981), 50(6): 740-746
Issue Date	1981-11-01
URL	http://hdl.handle.net/2433/208572
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

Kyoto University

Arch Jpn Chir 50(6), 740～746, Nov., 1981

Determination of Serum Tocopherols by High Performance Liquid Chromatography

HITOSHI KATO, HIROSHI TANIMURA and YORINORI HIKASA

The 2nd Department of Surgery, Faculty of Medicine, Kyoto University
(Director: Prof. YORINORI HIKASA)
Received for Publication, Sept. 8, 1981.

Abstract

A simple and rapid method was desired to determine all the analogues of Vitamin E; α-, β-, γ- and δ-tocopherols in human serum with high performance liquid chromatography. Tocopherols, and tocol as an internal standard, were extracted into n-hexane and injected onto a Nucleosil-5NH_2 column with a mobile phase of 45/55 (by vol.) isopropyl ether/n-hexane. The eluted compounds were detected by a fluorescence spectrophotometer and calculated according to peak-height ratios to the internal standard. The time required was less than 10 minutes. The within-day precisions for all tocopherols were less than 2.1%, and the recoveries from fortified serum were from 94% to 100%. The average levels of α-, β-, γ-, δ- and total tocopherol in 19 sera of healthy Japanese were 9.1±1.7 mg/L, 0.1±0.1 mg/L, 0.8±0.4 mg/L, 0.0 mg/L, and 10.2±0.2 mg/L, respectively. This rapid method for complete separation of tocopherols was applied in various diseases to know any differance among them, and in surgical field to assess the influence of operation on serum tocopherol levels.

Introduction

Recent reports have pointed out the importance of Vitamin E as an antioxidant or membrane stabilizer[7,15]. It is necessary to determine each analogue of Vitamin E separately, as their biological activities differ greatly[2].

Many methods of determining tocopherols have been described. The routine fluorometric methods were simple, but the individual tocopherols could not be differentiated[12,13]. Thin-layer chromatography[10] and combined thin-layer/gas liquid chromatography[14] could separate them, but these methods are complex. Liquid-solid chromatography could separate them directly, but required considerable time[17].

High performance liquid chromatography has been used recently, and a few good results have been obtained[1,5,6,8]. However, more improvements were desired in respect to time required, separation of the analogues and reproducibility.

Key words: High performance liquid chromatography, Vitamin E, Intravenous fat emulsion.
索引語：高度液体クロマトグラフィー，ビタミンE，静注用脂肪乳剤.
Present address: The 2nd Department of Surgery, Faculty of Medicine, Kyoto University, Sakyoku, Kyoto, 606, Japan.

The present paper describes a rapid, more sensitive and completely separating method using high performance liquid chromatography equipment with a spectrofluorophotometer for detecting, with good reproducibility, the analogue of Vitamin E in serum.

Materials and methods

Apparatus

A Hitachi 635 type high performance liquid chromatography and a Hitachi 204S fluorescence spectrophotometer (Hitachi, Tokyo, Japan) were used, also a 4 mm i.d. × 250 mm stainless-steel column packed with 5 μm Nucleosil-5NH_2 (Macherey Nagel, West-Germany). The detector with a flow-through cell 2 mm in path width was monitored with 298 nm excitation and 325 nm emission. The peak heights and their ratios were calculated with a Digital integrator TR-2221 (Takeda Riken, Tokyo, Japan).

Reagents

The n-hexane used was a product prepared for pesticide residue analysis (Wako Pure Chemical, Osaka, Japan). Ethanol and isopropyl ether were guaranteed reagents obtained from Kanto Chemical, Tokyo, Japan. Isopropyl ether was washed with 10% NaOH to remove the antioxidation materials and then washed again with distilled water until the material was neutral. Pure α-, β-, γ- and δ-tocopherols, which were separately isolated from soy bean serum and purified by ion exchange chromatography, and tocol were donated by Eisai, Tokyo, Japan.

Method

0.5 ml of serum was added to 0.5 ml of distilled water in a 10 ml brown centrifuge tube with a glass-stopper and mixed well with a vortex mixer. Then 1.0 ml of ethanol containing 7 μg of tocol as an internal standard was added. After stirring, 5 ml of n-hexane was added and mixed with the mixer (160 rpm, 1 min.), and 4 ml of the supernatant was dried under nitrogen gas flow at 40°C in a water bath. The residue was redissolved in 50 μl of n-hexane and 20 μl was injected onto the column. The chromatographic conditions are shown in Table 1.

To get the standard curves, samples of known volume of α-, β-, γ- and δ-tocopherols ranging from 0.005 μg to 25 μg (representing 0.03 mg/L to 156 mg/L) were injected onto the column with an equal volume of tocol. Curves were drawn by plotting the peak height ratios of tocopherols to tocol against their weight ratios.

Table 1. Chromatographic conditions.

Gel;	Nucleosil-5NH_2 (5 μ)
Column;	4 i.d. × 250 mm
Column temperature;	Ambient
Mobile phase;	Isopropyl ether: n-Hexane (45 : 55)
Flow rate;	1.3 ml/min.
Pressure;	75 kg/cm^2
Detector;	Hitachi 204S fluorescence spectrophotometer (Ex. 298 nm, Em. 325 nm)

Results and discussion

The chromatogram obtained for standard solutions containing 5.95 μg of α-, 5.78 μg of β-, 5.50 μg of γ-, and 4.98 μg of δ-tocopherol and 6.73 μg of tocol is shown in Figure 1. Each tocopherol separated distinctly, and all the analogues were detected within 10 minutes. The detection limits were 0.06, 0.06, 0.07 and 0.07 mg/L for the four tocopherols respectively. Linear relationships between peak-height ratios and mass ratios were noted up to 60 mg/L. At higher concentrations, the slopes of the lines decreased. The regression lines were as follows; α-tocopherol $y=3.324x+0.095$, β-tocopherol $y=4.794x+0.027$, γ-tocopherol $y=4.794x+0.072$, δ-tocopherol $y=3.600x+0.027$.

The chromatogram of serum tocopherols of a healthy male is shown in Figure 2. The within-day precisions were 1.6%, 1.5% and 1.3%, for α-, β- and γ-tocopherol respectively (in 10 consecutive injections of normal serum, the mean and SD of the tocopherols were 9.7 ± 0.2, 0.1 ± 0.0, 1.0 ± 0.0 and 0.0 mg/L).

The day-to-day precisions were 3.3%, 4.4% and 4.6% (the same serum). Analytical recoveries for fortified serum were from 94 to 100% (Table 2).

Nineteen sera of healthy adults were analysed by this method (Table 3). α-Tocopherol, which has the highest biological activity, accounted for about 90% of the total tocopherol, β- for 1–2%, and γ- for 8%; δ-tocopherol was not detected. This proportion is consistent with that of former reports[3,4].

The serum levels of α-tocopherol in various diseases are shown in Figure 3. Patients with

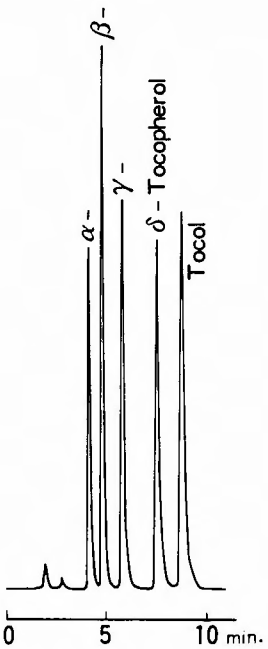

Fig. 1. Chromatogram of standard solution containing α-, β-, γ-, δ-tocopherols and tocol

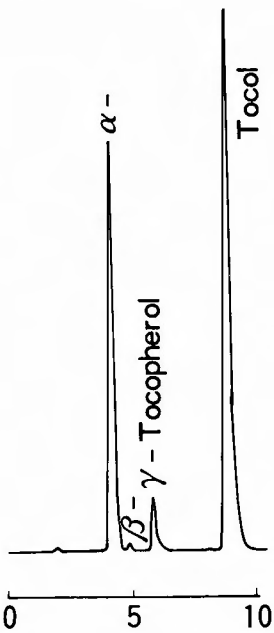

Fig. 2. Chromatogram of serum tocopherols in a healthy male

pancreatic diseases, such as pancreatic cancer and pancreatitis, had lower α-tocopherol (5.7±1.2 mg/L) and total tocopherol (6.2±1.3 mg/L) levels. In biliary atresia very low values were seen; α-tocopherol 2.3±1.4 mg/L and total tocopherol 2.9±0.6 mg/L. The patients with biliary atresia were infants, and it has been reported that new-born babies and infants have lower levels of

Table 2. Recovery of each tocopherol.

	Serum Toc. A (μg)	Added Toc. B (μg)	Total Toc. A+B (μbg)	Observed Toc. C (μg)	Recovery Toc. (C-A)/B (%)
α-Toc.	4.76	1.50	6.26	6.26	100
β-Toc.	0.10	1.24	1.34	1.26	94
γ-Toc.	1.14	1.36	2.50	2.44	96
δ-Toc.	0.00	1.04	1.04	1.00	96

Table 3. Serum tocopherols in normal subjects (n=19).

Tocopherol	Concentration (mg/L)	
	Range	Mean
α-Toc.	6.7-11.9	9.1
β-Toc.	0.1- 0.2	0.1
γ-Toc.	0.2- 1.3	0.8
δ-Toc.	0.0- 0.0	0.0
total	7.8-12.2	10.2

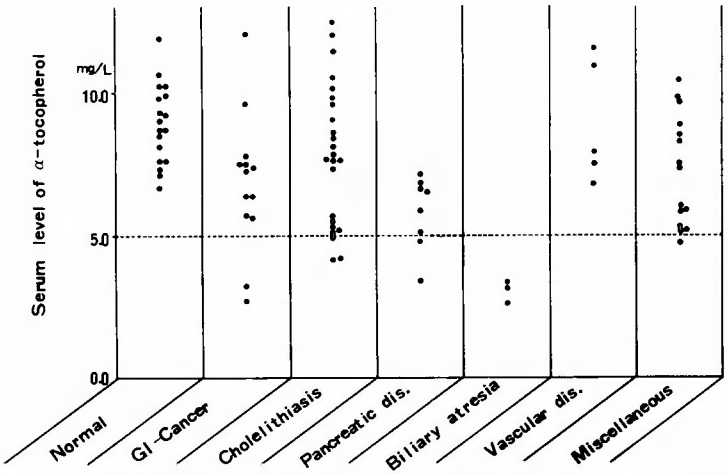

Fig. 3. Serum levels of α-tocopherol in various diseases

Vitamin E than adults[16], but the main reason for these low values seems to be a disturbance in the absorption of fat soluble vitamins.

Patients who received soybean fat emulsions intravenously showed elevations of Vitamin E, especially γ- and δ-tocopherol. This fat emulsion contains 20–30 mg/500 ml of Vitamin E, and 60–70% of that is γ-, 25–35% is δ-tocopherol, and less than 5% is α- and β-tocopherols[9,11]. These tocopherols are derived from soy-bean oil. After the cessation of intravenous infusion, the γ- and δ-tocopherol levels decreased simultaneously and reached normal levels within a few days.

The patients who underwent abdominal operation showed 20% decrease of serum α-tocopherol in average in the early post-operative days (Fig. 4). This decrease might be caused by fasting or bleeding, but it seems to be most reasonable to think that consumption of platelet plays a major role in such great decrease.

After ABE and colleagues first separated all tocopherols by high performance liquid chromatography, several methods using this chromatography have been reported. However, they all need to be improved at two analytical points. Most of them took more than 17 minutes, which is too long for the determination of many samples, and in others the separation of each tocopherol was not adequate.

With the present method Vitamin E analogues were almost completely separated within 10 minutes, and the reproducibilities were satisfactory; therefore this method is suitable for routine use and for clinical studies.

References

1) Abe K, Yuguchi Y, et al: Quantitative determination of tocopherols by high-speed liquid chromatography. J Nut Sci Vitaminol **21**: 183, 1975.
2) Bieri JG, Farrell PM: Vitamin E. Vit. and Hormone **34**: 31, 1976.
3) Bieri JG, Prival EL: Serum Vitamin E determination by thin-layer chromatography. Proc Soc Exp Biol Med **120**: 554, 1965.
4) Chow CK: Distribution of tocopherols in human plasma and red blood cells. Am J Clin Nutr **28**: 756, 1975.

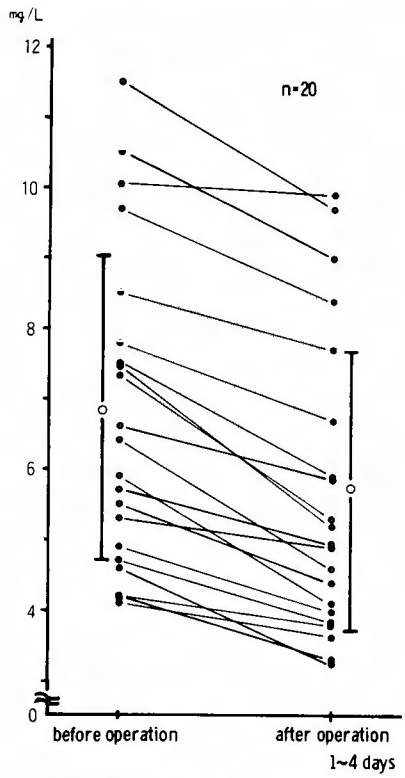

Fig. 4. Serum level of α-tocopherol before and after operation

5) De Leenheer AD, De Bevere VOPC, and Claeys AE: Measurement of α-, β-, and γ-tocopherol in serum by liquid chromatography. Clin Chem **25**: 425, 1979.
6) Eriksson T, Sorensen B: High-performance liquid chromatography of Vitamin E. Acta Pharm Suec **14**: 475, 1977.
7) Hafeman DG, Hoekstra WG: Lipid peroxidation in vivo during Vitamin E and Selenium deficiency in the rat as monitored by ethane evolution. J Nutri **107**: 666, 1977.
8) Jansson L, Nilsson B, et al: Quantitation of serum tocopherols by high-performance liquid chromatography with fluoresence detection. J Chromatogr **181**: 242, 1980.
9) Kato H, Tanimura H, et al: Serum level of Vitamin E family in surgical diseases. Jap J Surg Metab Nutri **14**: 272, 1980.
10) Stowe HD: Separation of β- and γ-tocopherol. Arch Biochem Biophys: **103**: 42, 1963.
11) Tanimura H, Kato H and Hikasa Y: Clinical significance of tocopherols analysis in serum. Proceedings of the Symposium on Chemical Physiology **19**: 148, 1979.
12) Taylor SL, Lamden MP and Tappel AL: Sensitive fluorometric method for tissue tocopherol analysis. Lipids **11**: 530, 1976.
13) Thompson JN, Erdody P and Maxwell WB: Simultaneous fluorometric determination of Vitamins A and E in human serum and plasma. Biochem Med **8**: 403, 1973.
14) Lovelady HG: Separation of individual tocopherols from human plasma and red blood cells by thin-layer and gas-liquid chromatography. J Chromatogr **85**: 81, 1973.
15) Maggio B, Diplock AT and Lucy JA: Interaction of tocopherols and ubiquinones with monolayers of phospholipids. Biochem J **161**: 111, 1977.
16) Mino M, Nishida Y, Kijima Y, et al: Tocopherol level in human blood cells. J Nutr Sci Vitaminol **25**: 505, 1979.
17) Niekerk PV: The direct determination of free tocopherols in plant oils by liquid-solid chromatography. Anal Biochem **52**: 533, 1973.

和文抄録

高速液体クロマトグラフィーによる血清トコフェロール同族体の測定

京都大学医学部外科学教室第2講座（主任：日笠頼則教授）
加藤 仁司，谷村 弘，日笠 頼則

ヒト血清中ビタミンE同族体，即ち α, β, γ, δ-トコフェロール測定の為，簡便で迅速な測定方法が望まれていた．今回，我々はトコールを内部標準として，ビタミンE同族体をn-ヘキサン中に抽出し，ヌクレオシルー $5NH_2$ (5μ) カラムを使用し，イリプロピルエーテル・n-ヘキサン (45:55) を移動相とする高速液体クロマトグラフィーを開発した．検出は螢光光度計にて行ない，内部標準との波高比にて濃度を計算した．測定時間は10分以内で，日内変動は2.1%以下，添加回収率は94～100%であった．この測定方法を応用して，1) 健常人19名における α, β, γ, δ 及び総トコフェロール値は，平均 9.1±1.7 mg/L, 0.1±0.1 mg/L, 0.8±0.4 kg/L, 0.0 kg/L, 10.2±0.2 mg/L であった．2) 種々の疾患における血清中のトコフェロール値を測定した結果，特に膵疾患，胆道閉鎖症をおいてα-トコフェロールが著明に減少していた．3) 脂肪乳剤上には 20-30 mg/500 ml の総トコフェロールが含まれ，60～70%はγ, 25～35%はδで5%以下がα及びβ-トコフェロールであった，その投与により，血清中のγ及びδ-トコフェロールの上昇も認められた．4) 手術侵襲によるα-トコフェロールの減少は平均20%である．等の結果が得られ，臨床応用に十分役立つ測定法であるといえよう．